## REMARKS

Entry of the foregoing and reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested in light of the remarks which follow.

As correctly noted in the Office Action, Claims 32-51 and 53-58 are pending. Only Claim 42, 53 and 59 are currently under consideration. The remaining claims stand withdrawn as directed to non-elected subject matter.

Although Applicant traversed the restriction, Applicant now cancels all claims drawn to non-elected subject matter (*i.e.,* Claims 32-41, 43-51, and 54-58). Applicant introduces new Claims 60-72. The subject matter of the new claims can be located at least in the claims as originally filed.

Applicant has amended Claims 42, 53 and 59. The amendments more distinctly claim the subject matter of the invention and do not introduce any prohibited new matter. The amendments to the claims are supported at least by claims as originally filed. Applicant reserves the right to file continuations and divisionals on any of the subject matter canceled by way of this or any Amendment.

Applicants further note that the Examiner has identified U.S. Pat. Nos. 5,851,832 and 5,980,885 and made them both of record but found the claims to be free from this art. Likewise, the article of Ajo *et al.*, "Growth Hormone Action on Proliferation and Differentiation of Cerebral Cortical Cells from Fetal Rat," *Endocrinology* 144(3) 1086-97 (2003) (published after Applicant's filing date) was made of record and the claims were found to be free of this reference.

### 1. Oath and Declaration

The Examiner objected to the oath and declaration due to a typographical error of placement of an "X" in the "No" box on the first page of the Declaration. Submitted herewith is a substitute executed Declaration correcting that defect. Accordingly, the objection can be withdrawn.

Applicant notes for the record that the placement of the "X" was a typographical error. It is clear from the International PCT papers that the Applicant was claiming priority to and benefit of his Swedish application. Provision of the newly executed Oath and Declaration merely corrects a typographical error.

**2. Drawings**

The Examiner objected to the drawings as failing to comply with 37 C.F.R. § 1.84(p)(5). Applicant has prepared new figures to comply with the rules for drawings and has amended the text at pages 10 and 14 to correctly reflect the use of the symbols in the figures. This amendment does not introduce any prohibited new matter as the change would be apparent to an artisan of ordinary skill. Accordingly, Applicant respectfully requests withdrawal of the objection in light of the above amendments and statements.

**3. Objections**

The Examiner objected to Claim 53 for depending from a canceled claim, Claim 52. Applicant has amended Claim 53 to depend from pending Claim 59 thereby obviating the objection. Accordingly, Applicant respectfully requests withdrawal of the objection.

**4. Claim Objections Under 35 U.S.C. § 112, First Paragraph - Enablement**

A. Claims 42, 53 and 59

Claims 42, 53 and 59 stand rejected as lacking enablement for recitation of the phrase "functionally equivalent analog." Without acquiescing as to the merits supporting this rejection, Applicant has amended the Claims by deleting the phrase without prejudice of or disclaimer to the subject matter. Applicant reserves the right to file continuation or divisional applications on the canceled subject matter. By this amendment, the rejection is obviated and accordingly should be withdrawn.

B. Claims 53 and 59

Claims 53 and 59 further stand rejected for lacking enablement based on the specification's purported failure to provide a "successful showing that the growth hormone treatment actually induced lineage determination or neurogenesis". (Office Action, p. 6, para. 15). The Examiner goes on to assert that incorporation of BrdU is a non-specific test of self-proliferation. Therefore, the results "could be due to self-proliferation of glia such as microglia, astrocytes, oligodendrocytes, and not neurons *per se*." (Office Action, pp. 6-7). In paragraphs 16-22 of the Office Action, the Office expounds on the various *Wands* factors, asserting that due to unpredictability of the nature of the technology, the claims lack enablement. Specifically, the Examiner purportedly supports his position by providing several references which purportedly teach: (1) that numerous obstacles exist to successful stem cell therapy and discuss the unpredictability of the fate of neuron stem cells transplanted into a patient (Rossi and Cattaneo, May 2002); (2) the problem of using non-recombination or chemically synthesized growth hormone (Ellis, 1992); (3) that issues such as restoration of circuitry, integration into existing structures, and repair/replacement of lost neuronal function must be taken into consideration and thus adds to the complexity and unpredictability of practicing this invention (Fricker-Gates *et al.*, 2001); and (4) that the factors of the donor's age, host's age, availability of neurotropic factors in the host and donor tissue, immunological response, target donor matching and vascularization as taught by Gage *et al.* further shows unpredictability. The above references are used to allegedly support the assertion that there is insufficient guidance to overcome these obstacles or counteract the level of unpredictability in the art of neuronal cell transplantation.

Applicant respectfully traverses the rejection. "As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied." M.P.E.P. § 2154.01(b). It would appear from the rejection, that the Office is raising the issue of "how to use" and not "how to make". Applicant allegedly provides insufficient disclosure regarding "how to make". With regard to "how

to use", the Office appears to raise the standard of patentability to prove safety and efficacy in humans. Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans. Were [the PTO] to require Phase II testing in order to prove utility, the associated costs would prevent many companies from obtaining patent protection on promising new inventions, thereby eliminating an incentive to pursue, through research and development, potential cures in many crucial areas. *In re Brana*, 34 U.S.P.Q.2d 1436, at 1442-43 (Fed. Cir. 1995). Thus, the fact that Applicants have not moved their invention to the stage of human testing is not fatal. It is sufficient to show that the disclosed differentiation and proliferation response obtained *in vivo* may be achieved in the appropriate animal model.

Applicant has provided *in vivo* testing in animals. Such testing is not required to file a patent application. Patent applications can be filed without any examples or data, thereby forming a ***constructive reduction to practice*** of the invention. An applicant need not have actually reduced the invention to practice prior to filing. M.P.E.P. § 2164.02. Nevertheless, Applicant in the instant case has provided working examples and data which evidence that neurogenesis is achieved by the administration of growth hormones (GH).

Further, the Examiner asserts that the Example provided in the application is a non-specific test of cell proliferation, because BrdU incorporation is a non-specific test and thus the observed result could be due to cell proliferation of glia and not due to proliferation of neurons *per se*. Applicant respectfully disagrees with this conclusion. Applicant have performed experiments which demonstrate that growth hormone (GH) treatment results in an increase in neural stem cell proliferation and not an increase in glia cell proliferation.

Applicant tested co-localization of BrdU immunoreactivity with the immunoreactivity of the neuronal cell markers, Calbindin $D_{28K}$ and NeuN as compared to the localization of the glial/astrocyte marker, GFAP. Applicant observed that the Calbindin $D_{28K}$- and BrdU-immunoreactive cell fractions was $41\% \pm 4\%$ in the GH treated animals and

43% ±5% in the untreated control animals. In looking at co-localization of the GFAP and BrdU markers, 17% ±3% of the BrdU-cells treated with GH were also GFAP positive as compared to 19% ±4% in the untreated control cells. To confirm that the increase of BrdU- and Calbindin $D_{28K}$-immunolabeled cells represented new neurons, Applicant performed additional double-immunolabeling characterizations using the neuronal marker, NeuN. Again, equal fractions of BrdU- and NeuN-labeled cells (*i.e.*, 54% ±5% and 55% ±4% respectively) were observed in the GH-treated animals as compared to the untreated controls.

Thus, considering that the number of newborn stem cell progeny was increased after GH treatment as discussed in the example of the instant application, and that the resulting fraction of neurons and glial cells were unaffected (as discussed above), it can be concluded that ***GH treatment increases neurogenesis through an increase in neural stem cell proliferation***. The materials and methods utilized to obtain the above discussed data is provided in the attached appendix. In the event that the Examiner requires this information to be submitted in the form of a Declaration under 37 C.F.R. § 1.132, the Examiner is invited to contact the undersigned attorney and such a Declaration shall be prepared and submitted.

Accordingly, the specification provides to the skilled artisan sufficient detail to practice the claimed invention. In view of the above arguments and the amendments to the claims, Applicant asserts that claimed invention is clearly enabled and respectfully requests withdrawal of the rejection and allowance of the claims.

**5. Rejections Under 35 U.S.C. § 112, Second Paragraph**

Claims 53 and 59 stand rejected under 35 U.S.C. § 112, second paragraph, as purportedly incomplete for omitting essential elements. The Examiner stated that the essential elements were the reason for transplanting the cells into the patient and where the transplant should occur.

Applicant has amended the claims to recite "patient in need of neuron regeneration". Recitation of this phrase sufficiently identifies the metes and bounds of the invention. Location of the transplantation may vary by patient as would be evident to an artisan of ordinary skill. Accordingly, in view of the above amendments and arguments, the rejection under § 112, second paragraph has been obviated. The rejection can thus be withdrawn and the claims allowed.

**6. Claim Rejections Under 35 U.S.C. § 102**

A. Swedish Patent Application No. 9804064-A

Claims 42, 53 and 59 stand rejected under 35 U.S.C. § 102(a) as purportedly anticipated by Swedish Patent Application 9804064-A. Applicant notes that this application is Applicant's priority document. With the submission of the re-executed Oath and Declaration, this rejection is moot.

B. Almazan *et al.*

Claim 42 stands rejected also under 35 U.S.C. § 102(b) as purportedly anticipated by Almazan *et al.*, *Develop. Brain Res.* 21: 257-64 (1985).[1] Almazan is alleged to teach "a method of growing fetal cells derived from the telencephalon of rats in the presence of bovine growth hormone thus meeting limitations of claim 42". (Office Action, pp. 10-11).

Applicant respectfully traverses the rejection. Without acquiescing as to the merits of the grounds for rejections, Applicant has amended claim 42 to recite stem cells and progenitor cells. Such cells are known to be immature cells and would not be characterized as neurons or glial cells. The Almazan reference only refers to potential proliferation and differentiation of neurons and glial cells in cultures of cell aggregates derived from rat telencephalon.

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[1] Applicant notes for the record that the correct cite is presented herein. The Office Action presents the cite as Almazan *et al.*, (August 1985), *Developmental Brain Research* **353(2)**: 257-264. *See* Office Action, p. 10, para. 25.

**Page 257:** "Neurons and glial cells within the aggregates proliferate and differentiate in culture..."

**Page 258:** "Aggregating cell cultures were prepared from....fetal rat. . . telencephalons...."

Such cell aggregates do not read upon Claim 42, as amended which refers to progenitor and stem cells and not cell aggregates. Accordingly, as the reference does not teach or suggest Claim 42 as amended, the rejection should be withdrawn and the Claim allowed.

C. Weiss *et al.*

The Examiner also rejected claim 42 under 35 U.S.C. § 102(b) as anticipated by U.S. Patent No. 5,750,376 issued to Weiss *et al.* in May 1998. The Examiner asserts that this patent allegedly discloses an *in vitro* method for proliferation and differentiation of neural stem cells and stem cell progeny. The paragraph cited in the Office Action is reiterated below (Col. 10, l. 58 to Col. 11, l. 11):

> This invention provides in one aspect a composition for inducing the proliferation of a multipotent neural stem cell comprising a culture medium supplemented with at least one growth factor, preferably epidermal growth factor or transforming growth factor alpha.
>
> The invention also provides a method for the in vitro proliferation and differentiation of neural stem cells and stem cell progeny comprising the steps of (a) isolating the cell from a mammal, (b) exposing the cell to a culture medium containing a growth factor, (c) inducing the cell to proliferate, and (d) inducing the cell to differentiate. Proliferation and perpetuation of the neural stem cell progeny can be carried out either in suspension cultures, or by allowing cells to adhere to a fixed substrate. Proliferation and differentiation can be done before or after transplantation, and in various combinations of in vitro or in vivo conditions, including (1) proliferation and differentiation in vitro, then transplantation, (2) proliferation in vitro. transplantation, then further proliferation and differentiation in vivo, and (3) proliferation in vitro, transplantation and differentiation in vivo.

The other section cited in the Office Action is located at Col. 20, ll. 40-56 which purportedly recited that the growth factor can be a growth hormone. Applicants also point to Example 11 of the Weiss patent, wherein it is stated that EGF and FGF-2 are growth hormones. *See* Col. 41, ll. 17-18.

Applicant respectfully traverses the rejection. "Anticipation requires the presence in a single prior art disclosure of all elements of a claimed invention arranged as in the claims". *Jamesbury Corp. v. Litton Industrial Products, Inc.*, 225 U.S.P.Q. 253, 256 (Fed. Cir. 1985). Applicant asserts that the skilled artisan would not conclude that the Weiss patent teaches the use of a growth hormone to differentiate and proliferate pluripotent or stem cells. Growth hormone is another name for somatotropin. *See* MeSH definition for "growth hormone" obtained from the NCBI website (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=mesh). As such, growth hormone is not categorized as a growth factor, nor does the list of growth factors list growth hormone as a member (*see* attached MeSH lists for growth hormone and growth factor). Growth factors include such compounds as cyclins, endothelial growth factors, fibroblast growth factors, interleukins, maturation-promoting factor, and nerve growth factors. (*See* MeSH definition for growth factor and growth substances). Based on the usage of "growth hormone" in Example 11, it appears that Weiss *et al.* is using "growth hormone" to mean a synonym for growth factor and does not mean somatotropin. Thus, a skilled artisan would not conclude based on the teachings of the Weiss specification that a growth hormone (*i.e.*, somatotropin) can be used in the methods and compositions taught by Weiss *et al.* As a consequence, Weiss *et al.* does not teach every element of the instant claimed invention. Accordingly, the rejection should be withdrawn and the claim allowed.

## CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited. In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

In the event any further fees are due to maintain pendency of this application, the Examiner is authorized to charge such fees to Deposit Account No. 02-4800.

Respectfully submitted,

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Date: October 10, 2003

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## APPENDIX

### Phenotype Analysis of BrdU Positive Cells

Sections were treated for DNA denaturation, as described in the example (*see* pp. 10-14) in the instant application, and then incubated in TBS-TS for 30 minutes. Thereafter, they were incubated overnight at 4°C with rabbit anti-Calbindin $D_{28K}$ (1:500; SWant, Bellinzona, Switzerland), mouse NeuN (1:30; Chemicon, Temecula, CA), or rabbit anti-GFAP (1:500, Dako, Glostrup, Denmark) and rat anti-BrdU antibody (1:200, Harlan, Loughborough, United Kingdom). GFAP and Calbindin $D_{28K}$ were detected with Texas Red-conjugated anti-rabbit IgG (1:200 for GFAP and 1:100 for Calbindin $D_{28K}$; Jackson ImmunoResearch, West Grove, PA). Calbindin $D_{28K}$ was detected with Cy5-conjugated anti-rabbit IgG (1:150; Jackson ImmunoResearch). NeuN was detected with Texas Red-conjugated anti-mouse IgG (1:100; Jackson ImmunoResearch). BrdU was labeled with an FITC-conjugated anti-rat IgG (1:150; Jackson ImmunoResearch) for 2 hours at 37°C. Immunofluorescence was detected with a Nikon Diaphot fluorescence microscope and confocal laser-scanning microscopy using a Bio-Rad 1024 system (Hercules, CA) according to manufacturer's instructions.

The co-localization of BrdU was determined with cell-specific markers in the GCL (ganglion cell layer) in six to eight 40-$\mu$m-thick coronal sections taken 240 $\mu$m apart in each animal. For the neuronal marker Calbindin $D_{28K}$, 511 BrdU-positive cells were analyzed with respect to co-localization. For the neuronal marker NeuN, 748 BrdU-positive cells were analyzed. For the glial marker GFAP, 902 BrdU-positive cells were analyzed. All cell-counting procedures were blindly performed.

Co-localization of BrdU immunoreactivity with immunoreactivity of the granule cell marker Calbindin $D_{28K}$ and the astrocyte marker GFAP was investigated to determine the phenotype of progenitor cell progeny in the dentate gyrus after long-term growth hormone (GH) therapy compared with that of hx (*i.e.*, hypophysectomized) controls. Using confocal

microscopy, we were able to detect co-localization of BrdU with either Calbindin $D_{28K}$ or GFAP in the GCL.

In GH treated hx animals, the Calbindin $D_{28K}$- and BrdU-immunoreactive cell fraction was observed to be 41%±4% in GH treated and 43%±5% (NS) in hx controls. In addition, 17%±3% of BrdU-positive cells were also GFAP positive compared with 19%±4% in hx controls (NS). To confirm that the increase of BrdU- and Calbindin $D_{28K}$-immunolabeled cells represented new neurons, additional double-immunolabeling characterizations with the neuronal marker NeuN were performed. Equal fractions of BrdU- and NeuN-labeled cells (54%±5.0% and 55%±4.0%, respectively) were observed in GH-treated animals as compared with untreated controls.